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Gene Expression Profiling of Rat Hippocampus Following Exposure to the Acetylcholinesterase Inhibitor Soman

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Soman (*O*-pinacolyl methylphosphonofluoridate) is a potent neurotoxicant. Acute exposure to soman causes acetylcholinesterase inhibition, resulting in excessive levels of acetylcholine. Excessive acetylcholine levels cause convulsions, seizures, and respiratory distress. The initial cholinergic crisis can be overcome by rapid anticholinergic therapeutic intervention, resulting in increased survival. However, conventional treatments do not protect the brain from seizure-related damage, and thus, neurodegeneration of soman-sensitive brain areas is a potential postexposure outcome. We performed gene expression profiling of the rat hippocampus following soman exposure to gain greater insight into the molecular pathogenesis of soman-induced neurodegeneration. Male Sprague–Dawley rats were pretreated with the oxime HI-6 (1-(((4-aminocarbonyl)pyridinio)methoxyl)methyl)-2-((hydroxyimino)methyl)-pyridinium dichloride; 125 mg/kg, ip) 30 min prior to challenge with soman (180 μg/kg, sc). One minute after soman challenge, animals were treated with atropine methyl nitrate (2.0 mg/kg, im). Hippocampi were harvested 1, 3, 6, 12, 24, 48, 72, 96, and 168 h after soman exposure and RNA extracted to generate microarray probes for gene expression profiling. Principal component analysis of the microarray data revealed a progressive alteration in gene expression profiles beginning 1 h postexposure and continuing through 24 h postexposure. At 48 h to 168 h postexposure, the gene expression profiles clustered nearer to controls but did not completely return to control profiles. On the basis of the principal component analysis, analysis of variance was used to identify the genes most significantly changed as a result of soman at each postexposure time point. To gain insight into the biological relevance of these gene expression changes, genes were rank ordered by *p*-value and categorized using gene ontology-based algorithms into biological functions, canonical pathways, and gene networks significantly affected by soman. Numerous signaling and inflammatory pathways were identified as perturbed by soman. These data provide important insights into the molecular pathways involved in soman-induced neuropathology and a basis for generating hypotheses about the mechanism of soman-induced neurodegeneration.

Introduction

Soman is a potent organophosphonate cholinesterase inhibitor. Exposure to soman decreases neural acetylcholinesterase activity, resulting in increased levels of acetylcholine leading to a cholinergic crisis. This cholinergic crisis induces convulsions and seizures and can lead to death if left untreated (*1, 2*). The standard therapeutic regimen for soman exposure is the administration of (1) the muscarinic antagonist atropine sulfate to block the effects of excess acetylcholine, (2) an oxime to reactivate inhibited acetylcholinesterase, and (3) an anticonvulsant such as diazepam to inhibit convulsions and seizure activity (*1, 3*). Although this therapeutic regimen significantly increases survivability, the development of postexposure neurodegeneration is an important concern. It has been demonstrated that the development of soman-induced neurodegeneration is tightly linked to the onset of seizures (*3–8*). Thus, rapid administration of anticonvulsants is critical to avert soman-induced neuropathology. Soman-exposed rats that experienced up to 10 min of seizure activity prior to drug-induced seizure termination were free of neuropathology at 24 h postexposure (*4*). However, there was increasing frequency in the incidence of neuropathology in animals that experienced 20 or 40 min of seizure activity prior to drug-induced seizure termination (*4*). In guinea pigs, less than 70 min of seizure activity resulted in neuropathology 24 h after soman exposure (*5*). Except in circumstances in which an individual would have immediate access to therapeutic compounds, such as well-equipped military personnel deployed in a suspected chemical warfare scenario, the rapid administration of therapeutics cannot be assumed. This would likely be the case in a civilian exposure scenario, in which rapid access to therapeutics is problematic. Therefore, understanding the molecular pathogenesis of soman-induced seizures is important in developing potential neuroprotectants for soman-induced brain damage in cases where the standard treatment regimen is not rapidly administered. Oligonucleotide microarrays provide a means to globally survey molecular responses to a toxicant of interest (e.g., soman) across a variety of exposure parameters (for review see ref *9*). We performed gene expression profiling of the hippocampus, a soman-sensitive area of the brain involved in learning, memory, and spatial reasoning (*8*), following soman exposure to gain insight into the molecular basis for soman-induced neurodegeneration.

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Experimental Procedures

In Vivo Exposure to soman. Male Sprague−Dawley rats (250−350 g, Charles River Laboratories, Wilmington, MA) were utilized in these studies. Animals were housed individually in polycarbonate shoebox cages with corncob bedding and maintained in controlled temperature and humidity on a standard 12 h light/12 h dark cycle with free access to food and water. All animal experiments were approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the requirements or standards stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, Publication No. 85-23, 1996), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

The soman exposure paradigm utilized in this study was based on the model developed by Shih et al. (*10*) and McDonough et al. (*11*). This model was developed to screen neuroprotectant and anticonvulsant drugs in rats exposed to soman. In this model, 100% of the animals develop seizures (>6 h in duration) and neuropathology. Lethality is >60% at 24 h postexposure in the absence of any pharmacologic treatments, but is reduced to 30% at 24 h postexposure with treatment. Animals were pretreated with the oxime HI-6 (125 mg/kg, ip, obtained from the Walter Reed Army Institute of Research, Rockville, MD) 30 min prior to challenge with soman (180 μg/kg, sc; diluted with 0.9% sodium chloride; soman was obtained from the Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD). One minute after soman challenge, animals were treated with atropine methyl nitrate (2.0 mg/kg, im, Sigma-Aldrich, St. Louis, MO). Vehicle control animals ($n = 4$; 2 euthanized at 1 h, 1 euthanized at 12 h, and 1 euthanized at 24 h) received an equivalent volume of vehicle (0.9% sodium chloride), HI-6 and atropine. Naïve animals ($n = 3$) were also included in the study and received no treatments. Animals were monitored postexposure for clinical signs of intoxication (e.g., salivation, secretions, and lacrimation), and time to the appearance of convulsions was recorded (average time to convulsions, 7 ± 3 min; range, 3−14 min; $n = 31$). Animals were allowed to remain in the laboratory for observation and returned to the holding room at the end of the working day.

Brains were harvested immediately following deep anesthesia with an injection of sodium pentobarbital (65 mg/kg, ip) at 1, 3, 6, 12, 24, 48, 72, 96, and 168 h after the onset of convulsions. A sample size of $n = 3$ was used for all time points except 3 h ($n = 6$) and 72 h ($n = 4$). The hippocampus was dissected, snap frozen in cooled isopentane (−70 °C), and stored at −80 °C until use.

Microarray Procedures. All microarray experiments were performed to comply with Minimal Information About a Microarray Experiment (MIAME) protocols and details can be found at the Gene Expression Omnibus (GEO) accessible through GEO Series accession number GSE13428[1]. Affymetrix Rat 230 2.0 oligonucleotide arrays were used as the microarray platform (https://www.affymetrix.com/support/downloads/package_inserts/rat230_2_insert.pdf; Affymetrix, Santa Clara, CA). Frozen rat hippocampi were homogenized in Tri Reagent (Sigma-Aldrich Chemical Co., St. Louis, MO) and the total RNA extracted according to the manufacturer's protocol (http://www.sigmaaldrich.com/ sigma/bulletin/t9424bul.pdf). RNA was DNase I treated and further purified using RNeasy columns (Qiagen, Valencia, CA). The quality and amount of RNA was monitored throughout processing with an Agilent Bioanalyzer (Agilent, Palo Alto, CA) and a NanoDrop ND-1000 UV−vis spectrophotometer (Nanodrop Technologies, Rockland, DE). Purified RNA was used to prepare biotinylated target RNA, with minor modifications from the manufacturer's recommendations (http://www.affymetrix.com/support/technical/manual/expression _manual.affx) as described previously (*12*). Briefly, 10 μg of total RNA was used to generate first-strand cDNA by using a T7-linked oligo(dT) primer. After second-strand synthesis, in vitro transcription with linear amplification was performed using biotinylated nucleotides (BioArray RNA Amplification and Labeling System, Enzo Life Sciences, Farmingdale, New York, NY). The target cRNA generated from each sample was processed as per manufacturer's recommendation using an Affymetrix GeneChip Instrument System (http://www.affymetrix.com/support/technical/manual/expression_manual.affx) as described previously (*12*).

Microarray Data Analysis. Scanned output files from each array were inspected for quality control as described previously (*12*). Raw signal intensities were imported into Partek Genomics Suite v6.3 (Partek, St. Louis, MO) and normalized using the robust multiarray averaging (RMA[2]) algorithm (*13*). The RMA normalized data were analyzed by principal component analysis (PCA) to determine the primary sources of variability in the data. This information was used to determine grouping variables for analysis of variance (ANOVA). A set of genes with significantly altered expression levels based on p-values from the ANOVAs was imported into Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com) to identify biological functions, canonical pathways, and gene networks significantly affected by soman exposure ($p < 0.05$, Fisher's exact test). Biological functions are categories that genes are classified into based on their cellular or physiological role in a healthy or diseased organism. Genes may be classified into more than one biological function. A canonical pathway is a well established signaling or metabolic pathway that is manually curated on the basis of published literature. Canonical pathways are fixed prior to data input and do not change upon data input. Networks are distinct from canonical pathways in that they are built de novo from input data based on known molecular interactions identified in the published scientific literature.

Results

Soman Induces Changes in Hippocampal Gene Expression over Time. Hippocampi from soman-exposed and control rats were processed for oligonucleotide microarray analysis. Data from probed and scanned arrays were normalized using the RMA algorithm and then analyzed by PCA (Figure 1). PCA simplifies the task of identifying sources of relative variability in a high dimensional data set by reducing the dimensions, and therefore the complexity, of the data set. Each individual biological sample is represented by the points on the three-dimensional plot. The distance between any pair of sample points is a function of the relative similarity between the two sample points in high dimensional space. Sample points that are near each other on the plot are relatively similar in a large number of variables (i.e., expression level of individual genes). Conversely, sample points that are far apart in the plot are relatively different in a large number of variables. In the soman-exposed rat hippocampus data set, the primary sources of relative variability in the PCA were exposure conditions (i.e., naïve, vehicle-exposed, or soman-exposed) and postexposure time point (1, 3, 6, 12, 24, 48, 72, 96, or 168 h) (Figure 1). The naïve and

[1] The data discussed in this publication have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE13428.

[2] Abbreviations: ERK, extracellular signal-regulated kinase; EGF, epidermal growth factor; FGF, fibroblast growth factor; GFAP, glial fibrillary acidic protein; GM-CSF, granulocyte-macrophage colony stimulating factor; ICAM, intercellular adhesion molecule; IL, interleukin; MAPK, mitogen activated protein kinase; PPAR, peroxisome-proliferator activated receptor; PCA, principal component analysis; Q-PCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; RMA, robust multiarray averaging; SOCS, suppressor of cytokine signaling; TNF-α, tumor necrosis factor-α; TRK, tyrosine kinase receptor; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

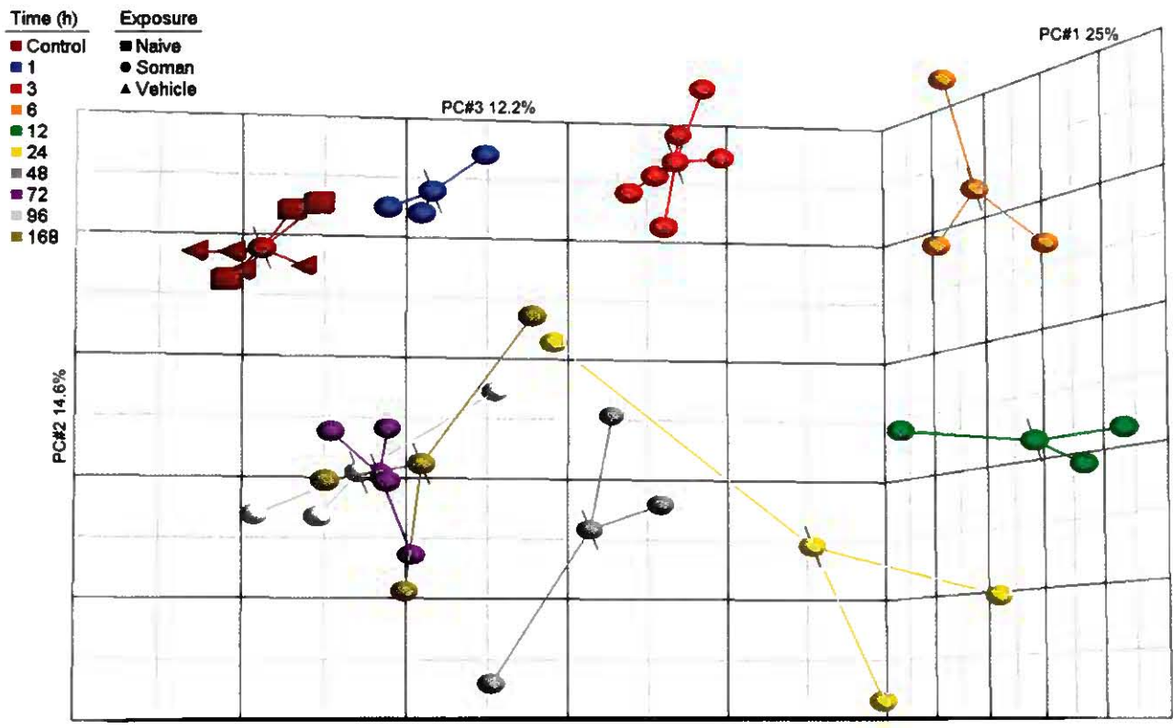

Figure 1. Principal component analysis (PCA) of hippocampal gene expression profiles from soman-exposed rats. Rat hippocampi were isolated at the indicated times after soman exposure and processed for oligonucleotide microarray analysis. The raw signal intensities were RMA transformed and visualized using PCA to determine the primary sources of variability in the data set. Point shape corresponds to exposure condition, and point color corresponds to the postexposure time point. The centroid of each time point group is shown as a sphere with an angled black line through its center. Each centroid is connected by vectors to each of the sample points in its corresponding time point group. Naïve controls were unexposed. Vehicle controls received all treatments except soman. Two vehicle control samples were collected at 1 h, one vehicle control sample was collected at 12 h, and one vehicle control was collected at 24 h. For simplicity in rendering the PCA, the time points for collection of each control sample were not identified in the PCA plot. The PCA plot rendered 51.8% of the total information content. Principal component 1 (PC1, *x*-axis) represents 25% of the information content of the PCA plot. PC2 (*y*-axis) represents 14.6% of the information content of the PCA plot. PC3 (*z*-axis) represents 12.2% of the information content.

vehicle control samples partition together (Figure 1, red symbols). The soman-exposed samples cluster by postexposure time point. The centroid of each time point group is shown as a sphere with an angled black line through its center. Each centroid is connected by vectors to each of the sample points in its corresponding time point group. For each time point group, the associated samples cluster together in an area within two standard deviations of its centroid. Sample groups 1 h, 3 h, 6 h, and 12 h partition progressively further away from the control sample group. Beginning with the 24 h sample group, the remaining sample groups partition progressively closer to the control sample group. The 72 h, 96 h, and 168 h sample groups partition closer to the controls than the other sample groups (with the exception of the 1 h sample group). However, none of the late time point groups partition with the control sample group. This suggests that even at 168 h after soman exposure the hippocampal gene expression profile has not returned to control levels.

Immediate and Early Biological Functions and Molecular Pathways Modulated by Soman Exposure: 1−3 h Postexposure. To examine the temporal response of the rat hippocampus to soman exposure, the data were filtered to individually isolate each postexposure time point, and a one-way ANOVA was performed with exposure (soman vs vehicle) used as the grouping variable for each time point. The genes most significantly modulated by soman exposure based on *p*-value cutoff were imported into Ingenuity Pathway Analysis to identify the biological functions and canonical pathways most significantly modulated by soman exposure at each postexposure time point. Significant gene expression changes were observed as early as 1 h after soman exposure ($p < 0.001$, Supporting Information, Table 1). A wide variety of biological functions were significantly modulated by soman, as revealed by gene ontology mapping of the soman responsive genes. The most significantly modulated biological functions at 1 h postexposure were gene expression, connective tissue disorders, immunological disease, inflammatory disease, skeletal and muscular disorders, and functions related to cell growth and death (cell death, cellular growth and proliferation, cell cycle, and cancer) (Supporting Information, Figure 1A). A number of canonical molecular pathways were significantly modulated by soman during the first hour postexposure. The most significantly modulated pathways were neurotrophin/tyrosine kinase receptor (TRK) signaling, p38 mitogen activated protein kinase (MAPK) signaling, extracellular signal-regulated kinase (ERK)/MAPK signaling, cAMP signaling, and interleukin-6 (IL-6) signaling (Supporting Information, Figure 1B). Interestingly, IL-6 expression was significantly upregulated by 3 h after soman exposure, peaked at 12 h postexposure, and did not return to control levels until 48−72 h postexposure (Figure 2). In silico construction of gene networks based on the most significantly modulated genes revealed that the highest scoring constructed network is centered around tumor necrosis factor-α (TNF-α) (Supporting Information, Figure 2). This is consistent with the significant and rapid upregulation of TNF-α expression as early as 1 h after soman exposure (Figure 3). At 3 h, many of the most significantly modulated biological functions are the same as those at 1 h, including connective tissue disorders, immunologi-

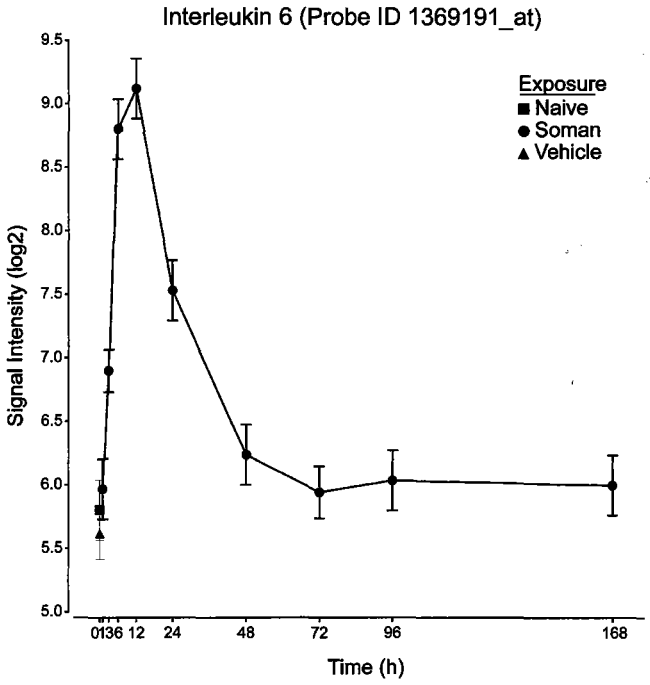

Figure 2. Soman exposure induces the expression of interleukin 6 in the hippocampus. Rat hippocampi were isolated at the indicated times after soman exposure and processed for analysis by oligonucleotide microarrays as described above. Soman exposure induces the expression of IL-6 by 3 h compared to that by naïve and vehicle controls. IL-6 expression peaks at 12 h postexposure and returns to control levels by 48−72 h postexposure.

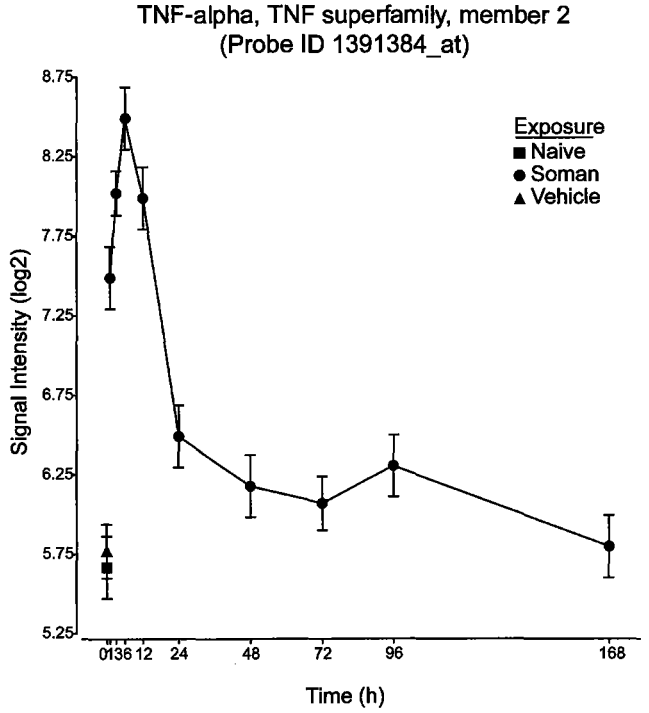

Figure 3. Soman exposure induces the expression of tumor necrosis factor-α (TNF-α; TNF superfamily, member 2) in the hippocampus. Rat hippocampi were isolated at the indicated times after soman exposure and processed for analysis by oligonucleotide microarrays as described above. Soman exposure induces the expression of TNF by 1 h postexposure compared to naïve and vehicle controls. TNF expression peaks at 6 h postexposure and returns to control levels by 168 h postexposure.

cal disease, inflammatory disease, skeletal and muscular disorders, and functions related to cell growth and death (cell death, cellular growth and proliferation, and cell cycle) (Supporting Information, Figure 3A; see Supporting Information, Table 2 for input genes, $p < 0.0001$). The canonical pathways most significantly modulated by soman include peroxisome-proliferator activated receptor (PPAR) signaling, IL-10 signaling, IL-6 signaling, neurotrophin/TRK signaling, and Toll-like receptor signaling (Supporting Information, Figure 3B). In addition, at 3 h after soman exposure, the MAP kinase signaling pathways are no longer among the most significantly modulated canonical pathways. As at 1 h, the highest scoring in silico constructed gene network at 3 h after soman exposure is centered around TNF-α (Supporting Information, Figure 4).

Biological Functions and Molecular Pathways Modulated by Soman Exposure: 6−24 h Postexposure. At 6 h postexposure, a large number of genes, biological functions, and canonical pathways continued to be significantly modulated by soman exposure (see Supporting Information, Table 3 for input genes, $p < 0.0001$). Behavior, nervous system development and function, cell cycle, gene expression, and functions related to cell and tissue function (connective tissue development and function, tissue morphology, cellular development and cellular movement) were the most significantly modulated biological functions (Supporting Information, Figure 5A). Neurotrophin/TRK signaling, valine leucine and isoleucine biosynthesis, IL-6 signaling, G-protein coupled receptor signaling, cAMP mediated signaling, IL-10 signaling, EGF signaling, and PPAR signaling were the most significantly modulated canonical pathways (Supporting Information, Figure 5B). At 12 h postexposure (see Supporting Information, Table 4 for input genes, $p < 0.0001$), gene expression, inflammatory disease, DNA replication, recombination, repair, and functions related to cellular growth and death (cellular growth and proliferation, cell cycle, cellular development, and cell death) were the most significantly modulated biological functions (Supporting Information, Figure 6A). IL-10 signaling, IL-6 signaling, acute phase response signaling, p38 MAPK signaling, death receptor signaling, Toll-like receptor signaling, nuclear factor-κ B (NF-κB) signaling, PPAR signaling, and neurotrophin/TRK signaling were the most significantly modulated canonical pathways (Supporting Information, Figure 6B). Interestingly, hippocampal IL-10 expression levels do not appear to be significantly altered by soman exposure over the time course studied, even though IL-10 signaling is the most significantly modulated canonical pathway (data not shown). At 24 h postexposure (see Supporting Information, Table 5 for input genes, $p < 0.001$), gene expression, cell death, connective tissue disorders, immunological disease, inflammatory disease, and functions related to the nervous system (nervous system development and function, behavior, and psychological disorders) were the most significantly modulated biological functions (Supporting Information, Figure 7A). Glucocorticoid receptor signaling, IGF-1 signaling, p53 signaling, PPAR signaling, IL-6 signaling, aminoacyl-tRNA biosynthesis, and epidermal growth factor (EGF) signaling were the most significantly modulated canonical pathways (Supporting Information, Figure 7B).

Biological Functions and Molecular Pathways Modulated by Soman Exposure: 48−96 h Postexposure. At 48 h postexposure (see Supporting Information, Table 6 for input genes, $p < 0.001$), dermatological diseases and conditions, and functions related to cell and tissue function (cell-to-cell signaling and interaction, tissue development, cell morphology, cellular development, cell death, cellular movement, and cellular growth and proliferation) were the most significantly modulated biological functions (Supporting Information, Figure 8A). Acute phase response signaling, IL-6 signaling, phospholipid degradation, coagulation system, granulocyte-

macrophage colony stimulating factor (GM-CSF) signaling, glutamate metabolism, IL-10 signaling, glycerophospholipid metabolism, arginine and proline metabolism, and glycerolipid metabolism were the most significantly modulated canonical pathways (Supporting Information, Figure 8B). At 72 h postexposure (see Supporting Information, Table 7 for input genes, $p < 0.001$), similar to the 48 h postexposure time point, cell-related biological functions were among the most significantly modulated biological functions (cell morphology, cellular development, cellular movement, cancer, cell-to-cell signaling and interaction, tissue development, cell death, cellular function and maintenance, and immune response) (Supporting Information, Figure 9A). The complement system, ephrin receptor signaling, acute phase response signaling, apoptosis signaling, integrin signaling, IL-10 signaling, N-glycan degradation, glycerophospholipid metabolism, glycosaminoglycan degradation, and phospholipid degradation were the most significantly modulated canonical pathways (Supporting Information, Figure 9B). At 96 h postexposure (see Supporting Information, Table 8 for input genes, $p < 0.001$), cellular functions (cellular movement, cell-to-cell signaling and interaction, cellular function and maintenance, cell death, and tissue development), immune system functions (immune response, immune and lymphatic system development and function, inflammatory disease, and immunological disease), and hematological system development and function were the most significantly modulated biological functions (Supporting Information, Figure 10A). The complement system, acute phase response signaling, ephrin receptor signaling, GM-CSF signaling, ERK/MAPK signaling, interferon signaling, IL-6 signaling, antigen presentation pathway, and the one carbon pool by folate pathway were the most significantly modulated canonical pathways (Supporting Information, Figure 10B).

Biological Functions and Molecular Pathways Modulated by Soman Exposure: 168 h Postexposure. At 168 h, the latest time point examined in this study, there was still significant modulation of gene expression due to soman exposure (see Supporting Information, Table 9 for input genes, $p < 0.001$). Gene functions (gene expression, RNA damage and repair, RNA post-transcriptional modification, and genetic disorder), cell and tissue functions (cellular development, connective tissue development and function, and cell-to-cell signaling and interaction), neurological disease, and reproductive system development and function were the biological functions most significantly modulated by soman (Supporting Information, Figure 11A). p38 MAPK signaling, glutamate metabolism, acute phase response signaling, alanine and aspartate metabolism, interferon signaling, hepatic fibrosis/hepatic stellate cell activation, complement system, fibroblast growth factor (FGF) signaling, and D-glutamine and D-glutamate metabolism were the most significantly modulated canonical pathways (Supporting Information, Figure 11B). Interestingly, the highest scoring in silico constructed gene network contained p38 MAPK as an important node (Supporting Information, Figure 12).

Discussion

Soman is a potent neurotoxicant that can induce rapid brain injury, even when medical treatments are administered. To understand in greater detail the molecular neural response to soman exposure, we performed microarray analysis of hippocampal tissue isolated from control and soman-exposed rats. We identified numerous soman-induced molecular changes. Early in the postexposure time course, the significant biological processes and canonical pathways appeared to generally follow the course of an inflammatory response (1−24 h postexposure), as suggested by biological processes such as immunological disease (1 h, 3 h, 24 h) and inflammatory disease (1 h, 3 h, 12 h, 24 h), and by canonical pathways such as p38 MAPK signaling (1 h, 12 h), Toll-like receptor signaling (3 h, 12 h), IL-6 signaling (1−24 h), and IL-10 signaling (3−12 h). In the middle to late portions of the time course (24−96 h), the significant biological processes and canonical pathways appeared to generally follow an injury response, as suggested by canonical pathways such as the acute phase response pathway (12 h, 48−168 h), coagulation pathway (48−72 h), and complement pathway (72−96 h). At the end of the time course, at 168 h, the significant biological processes and canonical pathways appeared to follow the course of a recovery response, as suggested by biological processes such as RNA damage and repair, connective tissue development and function, and cell-to-cell signaling and interaction, and canonical pathways such as glutamate metabolism, alanine and aspartate metabolism, D-glutamine metabolism, and D-glutamate metabolism. We observed temporal overlap of many of these processes and pathways.

Several of the changes we identified are confirmed by previous observations of soman-induced changes in gene expression reported in the literature, particularly increased expression of inflammatory mediators, such as IL-6 and TNF-α. Williams et al. (*14*) used the same exposure paradigm used in our current study and analyzed the expression of several inflammatory mediators by quantitative polymerase chain reaction (Q-PCR). They reported increases in IL-6, TNF-α, IL-1β, vascular cell adhesion molecule (VCAM), E-selectin, and intercellular adhesion molecule (ICAM) in the hippocampus following soman exposure. This confirms our observations that soman exposure induces the expression of IL-6 (Figure 2) and TNF-α (Figure 3). Additionally, we observed increases in IL-1β, VCAM, E-selectin, and ICAM consistent with Williams et al. (data not shown). To assay gene expression, Williams et al. utilized Q-PCR, which has a greater dynamic range compared to that of microarrays. Thus, the observed fold-change in gene expression is different for the two studies (for an example comparison of Q-PCR data and microarray data, see ref *12*). However, the trends in the changes in gene expression over time are similar in the two studies. While Williams et al. examined a limited number of time points following soman exposure (2, 6, 24, and 48 h), Dhote et al. (*15*) examined a wider range of time points (0.5, 1, 2, 6, 24, 48, and 168 h). They reported increases in IL-1β, IL-6, TNF-α, ICAM1, and also the suppressor of cytokine signaling (SOCS) 3 in mouse hippocampus following soman exposure (*15*). They also utilized Q-PCR to assay gene expression, and the changes in gene expression over time show similar trends compared to our data (data not shown).

While these studies, as well as ours, focused on changes in mRNA levels using Q-PCR or microarray techniques, recent work has also confirmed the upregulation of inflammatory mediators at the protein level as well. Svensson et al. identified increases in IL-1β following soman exposure at both the mRNA and protein levels using reverse transcription polymerase chain reaction (RT-PCR) and ELISA assays, respectively (*16, 17*). In addition, recent work by Johnson et al. (*18*) demonstrated soman-induced increases in TNF-α and IL-6 protein at the protein level, thus confirming our observations at the mRNA level as well as those of Williams et al. (*14*) and Dhote et al. (*15*).

Changes in neuroinflammatory mediators have also been observed in response to sarin, a similar organophosphonate nerve agent. Chapman et al. (*19*) reported increases in IL-1β, IL-6,

and TNF-α protein levels following sarin exposure in rats. The levels of expression of these inflammatory mediators were increased in rats that experienced 30 min of seizure activity compared to rats that experienced 5 min of seizure activity (*19*). Interestingly, sarin-induced changes in gene expression have also been examined at lower doses ($0.5 \times LD_{50}$ at 15 min and 2 h), and significant changes in these neuroinflammatory mediators were not reported (*20, 21*).

In addition to neuroinflammatory mediators, other aspects of our data are confirmed by other studies examining the pathogenesis of soman exposure. We identified complement system and coagulation system as canonical pathways significantly impacted by soman exposure 48−96 h postexposure (Supporting Information, Figures 8B, 9B, and 10B). This is consistent with studies reporting the formation of gliotic scarring following soman exposure (*22*). Collombet et al. identified increases in glial fibrillary acidic protein (GFAP) and vascular endothelial growth factor (VEGF) by immunohistochemistry in the hippocampi of soman-exposed mice during the time period of gliotic scar formation (*22*). We also observed significant increases in GFAP and VEGF mRNA levels after soman exposure (data not shown).

Taken together, our data are in agreement with a number of recent studies examining soman-induced changes in gene and protein expression in several model systems and exposure paradigms. This data set will serve to increase our understanding of the molecular response to soman exposure, during the acute toxic response, and during the neurodegenerative and recovery processes. Given that we see a gradual return to control gene expression profiles following soman exposure, it would be interesting in future studies to carry the time points out several weeks or months to examine the extent of recovery at the molecular level over a longer time frame. In addition, this data set will allow the generation of hypotheses related to soman-induced neurodegeneration and the identification of potential therapeutic targets for the development of neuroprotectant drugs that may serve as effective adjuncts to the current treatment regimen.

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Supporting Information Available: Tables of statistically significant genes for each time point, complete rankings of canonical pathways and biological functions for each time point, and selected network maps as referenced in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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